

It seems only yesterday when, in 1975, a bright star rose suddenly above the horizon of Separation Science. Three labs reported a two-dimensional (2-D, charge coupled orthogonally to mass separation) technique, for analysis of complex protein systems, simultaneously and independently, although most of the credit went just to O'Farrell [1–3]. Perhaps because his system was the most elaborate: in fact, he was able to resolve and detect about 1100 different proteins from lysed *E. coli* cells on a single 2-D map and suggested that the maximum resolution capability might have been as high as 5000 different proteins. Apart from the meticulous attention to detail, major reasons for the advance in resolution obtained by O'Farrell, compared to earlier workers, included the use of samples labeled with ^{14}C or ^{35}S to high specific activity, and the use of thin (0.8 mm) gel slabs for the second dimension, which could then be dried down easily for autoradiography. This detection method was able to reveal protein zones corresponding to one part in 10^7 of the sample (usually 1–20 μg was applied initially, since higher loads caused zone spreading, although up to 100 μg could be loaded). Coomassie blue, in comparison, was about 3 orders of magnitude less sensitive and could reveal only about 400 spots. For the first dimension, O'Farrell adopted gel rods of 13 cm in length and 2.5 mm in diameter. The idea was to run samples fully denatured, in what became known as the “O'Farrell lysis buffer” (9 M urea, 2 % Nonidet P-40, 2 % β -mercaptoethanol, and 2 % carrier ampholytes, in any desired pH interval). For the second SDS-PAGE dimension, O'Farrell [1] used the discontinuous buffer system of Laemmli [4] and, for improved resolution, a concave exponential gradient of polyacrylamide gel (usually in the intervals 9–15 or 10–14 %T, although wide porosity gradients, e.g., 5–22.5 %T, were also suggested). It is thus seen that, since its very inception, O'Farrell carefully selected all the best conditions available at the time; it is no wonder that his system was adopted as such in the avalanche of reports that soon followed (as for this writing, his paper has received about 20,000 citations!). O'Farrell 2-D mapping protocol became the basic methodology for what we would call today “proteomics” of any tissue or biological fluid, where thousands of components were suspected to be present. It remained the gold standard for such investigations at least for the following 25 years, up to the third millennium.

When I stated that the O'Farrell 2-D mapping introduced in 1975 had been a bright star I affirmed a widely held opinion, but it was not a polar star for us navigators in the starry sky represented by the polypeptides display in the 2-D gel. It was wishful thinking, at best. There was indeed a major impediment in this methodology, namely the erratic spot profile obtained by performing the first dimension in conventional IEF with soluble carrier ampholytes (CA), a la Svensson-Vesterberg, if you like [5]. There were no fixed stars in the firmament of 2-D maps: the apparent pI values kept changing, from batch to batch of CAs and, of course, from brand to brand, as manufactured by different companies (a chaotic synthesis, as you might remember) [6]. The situation was so frustrating that the Andersons recommended carbamylation train standards for mapping the pH gradient course and even preparing large volumes of stock solutions of CAs, obtained by carefully blending the various commercial products. The help was soon at hand, since in 1982 Bjellqvist et al. [7] launched another supernova in the sky of bioanalysis: immobilized pH gradients (IPGs), which were soon demonstrated to be able to overcome all these problems, while affording

86 exquisite resolution when run in narrow and ultra-narrow pH ranges. IPGs went largely
87 unnoticed for about a decade, even though they brought about some out-of-(terrestrial)
88 space results in bio-separations, including a resolution limit of $\Delta pI = 0.001$ for IPGs, vs. a
89 maximum resolving power of conventional IEF in soluble carrier ampholytes of $\Delta pI =$
90 0.01 , one order of magnitude less. Together with that, IPGs brought “democracy” for the
91 first time in electrokinetic processes. Up to their introduction, 2-D maps had been con-
92 ducted only in linear pH gradients, which penalize acidic proteins, jammed in the over-
93 crowded zone of the pH 4–6 region, where $>60\%$ of all proteins focus. Already in 1985,
94 we were able to describe a broad range, nonlinear IPG, strongly flattened in the crowded
95 region, with a sharp upward turn at alkaline pH values [8]; these ranges are by far the most
96 popular in today 2-D map analyses. Needless to say, IPGs proved to offer a loading ability
97 much superior to that of conventional IEF. Gels could be massively overloaded without
98 isoelectric precipitations or smears. This unique property could be exploited in 2-D maps
99 for detecting low-abundance proteins; whereas the typical protein load in 18×20 cm gel
100 slabs was up to 0.5 mg, with IPGs the load could be incremented up to 10 mg per gel [9]!

101 Two-dimensional maps represent only one half of the proteomics panorama of the AU4
102 present day (excluding from the count mass spectrometry, which of course has an enor-
103 mous significance in this field). The other half is a chromatographic approach developed by
104 Yates III and coworkers [10, 11], consisting of an online 2-D ion-exchange column
105 coupled to RP-HPLC, termed MudPit, for separating tryptic digests of 80S ribosomes
106 from yeast. The acidified peptide mixture is loaded onto a strong cation exchanger (SCX)
107 column; discrete eluate fractions are then fed onto a RP (reversed-phase) column, whose
108 effluent is injected directly into a mass spectrometer. This iterative process is repeated 12
109 times by using increasing salt gradient elution from the SCX bed and an increasing organic
110 solvent concentration from the RP beads (typically a C_{18} phase). In a total yeast lysate, the
111 MudPit strategy allowed the identification of almost 1500 proteins [11]. There are major
112 differences, though, between these two methods, in that the first one (2-D gel mapping)
113 consists in separating intact, proteins, as found in the original tissue in which they were
114 expressed, whereas in the second, chromatographic approach, only digested species are
115 analyzed, which means that subtle differences in expression (e.g., deamidation, proteolytic
116 cleavage products originating *in vivo*) are usually lost. Ideally, though, a lab should utilize
117 both approaches, since it is claimed that the advantage of MudPit (an unfortunate acronym
118 for such a powerful technique, since it literally means “hole full of mud”) would be the
119 ability of detecting also scarcely soluble membrane proteins and very acidic or basic species
120 whose pI values would be outside the range of IEF/IPG. It turns out, though, that a kind
121 of dichotomy developed, by which in the USA MudPit is mostly adopted (on the grounds
122 that it is mostly an instrumental approach, involving little labor from lab technicians)
123 whereas on this side of the Atlantic 2-D gel approaches are still much in vogue.

124 What should I state about the present book? In principle, there are so many books
125 already published describing in detail all methodological approaches, tips and hints on 2-D
126 gel slabs, that an additional one would seem to be pleonastic. Yet, by looking at the table of
127 contents and at the list of chapters (no less than 15), it is easy to note that this book is a very
128 special one in the panorama of manuals published up to the present. Whereas all the others
129 are “cookbooks” giving just about only recipes, this one, on the contrary, gives mostly and
130 perhaps only theory, a field too much ignored in all treatises on 2-D gel maps. So, I believe
131 that this is a most useful and unique approach, in that it would help users to avoid common
132 pitfalls due to ignorance of the basic theoretical mechanisms underlying the technique,
133 including data handling and proper tools for spot analysis. Of course potential users had



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